an adequate set of test concentrations for a definitive test.

(4) The EPA Environmental Research Laboratory in Gulf Breeze, Florida prepared a Research and Development Report entitled Acute Toxicity of Eight Drilling Fluids to Mysid Shrimp (*Mysidopsis bahia*), May 1984 EPA–600/3–84–067. The Gulf Breeze data for drilling fluid number 1 are displayed in Table 1 for purposes of an example of the probit analysis described above. The SAS Probit Procedure (SAS Institute, Statistical Analysis System, Cary, North Carolina, 1982) was used to analyze these data. The 96-hour LC50 adjusted for the estimated spontaneous mortality rate is 3.3 percent SPP with 95 percent limits of 3.0 and 3.5 percent SPP with the 1 to 9 dilution. The estimated spontaneous mortality rate based on all of the data is 9.6 percent.

TABLE 1—LISTING OF ACUTE TOXICITY TEST DATA (AUGUST 1983 TO SEPTEMBER 1983) WITH EIGHT GENERIC DRILLING FLUIDS AND MYSID SHRIMP

[fluid N2=1]

Percent concentration	Number exposed	Number dead (96 hours)	Number alive (96 hours)
0	60	3	57
1	60	11	49
2	60	11	49
3	60	25	35
4	60	48	12
5	60	60	0

V-C. The Partial Toxicity Test for Evaluation of Test Material

(1) A partial test conducted according to EPA protocol can be used economically to demonstrate that a test material passes the toxicity test. The partial test cannot be used to estimate the LC–50 adjusted for natural response.

(2) To conduct a partial test follow the test protocol for preparation of the test material and organisms. Prepare the control (zero concentration), one test concentration (3 percent suspended particulate phase) and the reference toxicant according to the methods of the full test. A range finding test is not used for the partial test.

(3) Sixty test organisms are used for each test concentration. Find the number of test organisms killed in the control (zero percent SPP) concentration in the column labeled X_0 of Table 2. If the number of organisms in the control (zero percent SPP) exceeds the table values, then the test is unacceptable and must be repeated. If the number of organisms killed in the 3 percent test concentration is less than or equal to corresponding number in the column labeled X_1 then the test material passes the partial toxicity test. Otherwise the test material fails the toxicity test.

(4) Data shall be reported as percent suspended particulate phase.

TABLE 2

X_0	X_1
0	22
1	22
2	23
3	23
4	24
5	24
6	25

VI. References

1. Borthwick, Patrick W. 1978. Methods for acute static toxicity tests with mysid shrimp (*Mysidopsis bahia*). Bioassay Procedures for the Ocean Disposal Permit Program, [EPA–600/9–78–010:] March.

2. Nimmo, D.R., T.L. Hamaker, and C.A. Somers. 1978. Culturing the mysid (*Mysidopsis bahia*) in flowing seawater or a static system. Bioassay Procedures for the Ocean Disposal Permit Program, [EPA–600/9–78–010]: March.

3. American Public Health Association et al. 1980. Standard Methods for the Examination of Water and Wastewater. Washington, DC, 15th Edition: 90–99.

4. U.S. Environmental Protection Agency, September 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA/600/4–90/027. Washington, DC, 4th Edition.

5. Finney, D.J. Probit Analysis. Cambridge University Press; 1971.

6. U.S. Environmental Protection Agency, May 1984. Acute Toxicity of Eight Drilling Fluids to Mysid Shrimp (*Mysidopsis bahia*). EPA–600/3–84–067.

APPENDIX 3 TO SUBPART A OF PART 435—PROCEDURE FOR MIXING BASE FLUIDS WITH SEDIMENTS

This procedure describes a method for amending uncontaminated and nontoxic (control) sediments with the base fluids that are used to formulate synthetic-based drilling fluids and other non-aqueous drilling fluids. Initially, control sediments shall be press-sieved through a 2000 micron mesh sieve to remove large debris. Then press-sieve the sediment through a 500 micron sieve to remove indigenous organisms that may prey on the test species or otherwise confound test results. Homogenize control sediment to limit the effects of settling that may have occurred during storage. Sediments should be homogenized before density determinations and addition of base fluid to control sediment. Because base fluids are

strongly hydrophobic and do not readily mix with sediment, care must be taken to ensure base fluids are thoroughly homogenized within the sediment. All concentrations are weight-to-weight (mg of base fluid to kg of dry control sediment). Sediment and base fluid mixing shall be accomplished by using the following method.

1. Determine the wet to dry ratio for the control sediment by weighing approximately 10 g subsamples of the screened and homogenized wet sediment into tared aluminum weigh pans. Dry sediment at 105 °C for 18–24 h. Remove sediment and cool in a desiccator until a constant weight is achieved. Reweigh the samples to determine the dry weight. Determine the wet/dry ratio by dividing the net wet weight by the net dry weight:

[Wet Sediment Weight (g)]/[Dry Sediment Weight (g)] = Wet to Dry Ratio [1]

2. Determine the density (g/mL) of the wet control or dilution sediment. This shall be used to determine total volume of wet sediment needed for the various test treatments.

[Mean Wet Sediment Weight (g)]/[Mean Wet Sediment Volume (mL)] = Wet Sediment Density (g/mL) [2]

3. To determine the amount of base fluid needed to obtain a test concentration of 500 mg base fluid per kg dry sediment use the following formulas:

Determine the amount of wet sediment required:

[Wet Sediment Density (g/mL)] × [Volume of Sediment Required per Concentration (mL)] = Weight Wet Sediment Required per Conc. (g) [3]

Determine the amount of dry sediment in kilograms (kg) required for each concentration:

{[Wet Sediment per Concentration (g)]/[Mean Wet to Dry Ratio]} × (1kg/1000g) = Dry Weight Sediment (kg) [4]

Finally, determine the amount of base fluid required to spike the control sediment at each concentration:

[Conc. Desired (mg/kg)] × [Dry Weight Sediment (kg)] = Base Fluid Required (mg) [5]

For spiking test substances other than pure base fluids (e.g., whole mud formulations), determine the spike amount as follows:

[Conc. Desired (mL/kg)] × [Dry Weight Sediment (kg)] × [Test Substance Density (g/mL)] = Test Substance Required (g) [6]

4. For primary mixing, place appropriate amounts of weighed base fluid into stainless mixing bowls, tare the vessel weight, then add sediment and mix with a high-shear dispersing impeller for 9 minutes. The concentration of base fluid in sediment from this mix, rather than the nominal concentration, shall be used in calculating LC_5. values.

5. Tests for homogeneity of base fluid in sediment are to be performed during the procedure development phase. Because of difficulty of homogeneously mixing base fluid with sediment, it is important to demonstrate that the base fluid is evenly mixed with sediment. The sediment shall be analyzed for total petroleum hydrocarbons (TPH) using EPA Methods 3550A and 8015M, with samples taken both prior to and after distribution to replicate test containers. Base-fluid content is measured as TPH. After mixing the sediment, a minimum of three replicate sediment samples shall be taken prior to distribution into test containers. After the test sediment is distributed to test containers, an additional three sediment samples shall be taken from three test containers to ensure proper distribution of base fluid within test containers. Base-fluid content results shall be reported within 48 hours of mixing. The coefficient of variation (CV) for the replicate samples must be less than 20%. If base-fluid content results are not within the 20% CV limit, the test sediment shall be remixed. Tests shall not begin until the CV is determined to be below the maximum limit of 20%. During the test, a minimum of three replicate containers shall be sampled to determine base-fluid content during each sampling period.

6. Mix enough sediment in this way to allow for its use in the preparation of all test concentrations and as a negative control. When commencing the sediment toxicity test, range-finding tests may be required to determine the concentrations that produce a toxic effect if these data are otherwise unavailable. The definitive test shall bracket the LC_5., which is the desired endpoint. The results for the base fluids shall be reported in mg of base fluid per kg of dry sediment.

References

American Society for Testing and Materials (ASTM). 1996. Standard Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing. ASTM E 1391–94. Annual Book of ASTM Standards, Volume 11.05, pp. 805–825.

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Suedel, B.C., J.H. Rodgers, Jr. and P.A. Clifford. 1993. Bioavailability of fluoranthene in freshwater sediment toxicity tests. Environ. Toxicol. Chem. 12:155–165.

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